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Research article

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Dynamics of change in broomrape populations (*Orobanche cumana* Wallr.) in Romania and Russia (Black Sea area)

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Abstract: The emergence of new broomrape populations (races) has been observed in the past 20 years in several countries (Romania, Moldova, Ukraine, Russia, Turkey, Bulgaria, Spain, Serbia, and China) where sunflower is frequently grown in the same sites without applying traditional crop rotations. Differential lines for sunflower broomrape races A to F have been secured. The new broomrape races have been identified by researchers as races G and H. The question of whether the same broomrape mutations can occur in one year and affect the same countries remains unsolved. Several results of new broomrape population emergences in some of the affected will be presented in the paper. A total of 390 genotypes were studied at four Romanian localities (Cuza Voda, Crucea-Stupina, Braila-Valea Canepii, and Tulcea-Agighiol) in 2014. At all four localities, a certain degree of sunflower broomrape infestation was observed in control hybrids and lines (Performer, LC-1093, LG-5661, and PR64LE20), which indicated the emergence of new populations higher than race H. The 390 studied genotypes had different reactions in all four localities. In 2015, 10 hybrids and controls were studied at five Romanian localities (Ciresu-Braila, Iazu-Ialomita, Stupina-Constanta, Topolog-Tulcea, and Viziru-Braila) and, according to the results, only hybrid Hy-7 was resistant in all localities. The results obtained from the three studied localities showed the emergence of new sunflower broomrape populations not controlled by

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gene for race H. Self-fertilization of hybrid Hy-7 produced the F_2 generation in 2016. In 2017, broomrape resistance was studied at the infested (contaminated) plot at the All-Russian Research Institute of Oil Crops by the name of Pustovoit V.S. – VNIIMK in Rostov on Don. The plot was found to be infested by new broomrape populations originating from Russia, Ukraine, Romania, Turkey, and Spain. The obtained results showed an infestation degree in 17.1% plants of hybrid Hy-7, 35% in the F_2 generation of Hy-7, control hybrids PR64LE25, LG-5580 and Donskoy-22 showed 19.4, 23, and 100% broomrape infestation, respectively. In conclusion, the plot contained broomrape populations which cannot be controlled by race H gene. According to the obtained results, a permanent change in variability of broomrape populations can be confirmed practically year after year. At present, new broomrape populations found at several localities are locally dispersed. Geneticists and breeders have to make joint efforts in further detailed studies of broomrape variability.

Keywords: broomrape; locality; new variability; population (race); sunflower.

Introduction

Broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production for more than a century. According to Morozov (1947), the first reports of broomrape in sunflower came from Saratov Oblast in Russia and date back to the 1890s. The same author mentions that the first sunflower varieties resistant to race A of *Orobanche* were developed by Plachek (1919) at the Saratov breeding station.

During the first half of the twentieth century, cultivars resistant to the prevailing broomrape races were created in several breeding centres of the former USSR. Six broomrape races (A, B, C, D, E, and F) were determined in the second half of the twentieth century. Genetics for resistance and differential lines of five races (A–E) were studied in detail by Vranceanu et al. (1980), while Pacureanu et al. (1998) studied race F (Figure 1).

Generally, broomrape (*O. cumana* Wallr.) causes economic damage in sunflower production in several countries around the world, especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, and China.

At the beginning of the twenty-first century, sunflower cultivation area suddenly expanded in many countries while the former crop rotation practices were discontinued, which led to sudden changes in broomrape populations, especially in Spain and the Black Sea area (Russia, Ukraine, Moldova, Romania, Turkey, Bulgaria). Changes in broomrape populations occurred in many countries during the past 15 years. New variability in broomrape populations has been determined as races G and H by several authors (Figure 2).

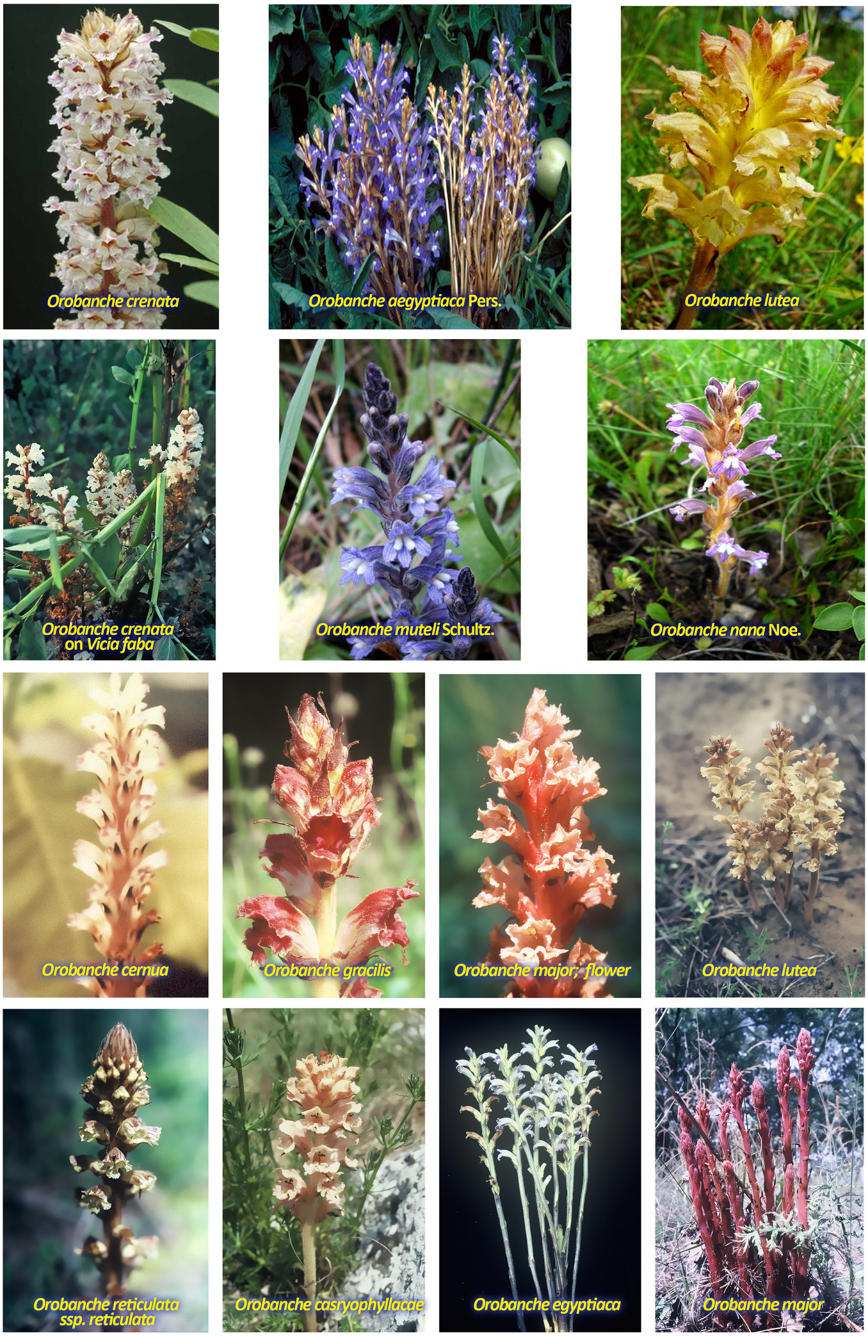

Figure 1: The morphological variability of the Broomrape population that can be found on various plant species in nature: (from left to right – *Orobanche cernua*; *Orobanche gracilis*; *Orobanche major*, flower; *Orobanche lutea*; *Orobanche reticulata* ssp. *reticulata*; *Orobanche caryophyllacea*; *Orobanche aegyptiaca*; *Orobanche major*; *Orobanche crenata*; *Orobanche aegyptiaca* Pers*;* *Orobanche lutea*; *Orobanche crenata* on *Vicia faba*; *Orobanche muteli* Schultz.; *Orobanche nana* Noe.) (The authors thank Dr. Horvath Zoltan for permission to use Orobanche's photographs).

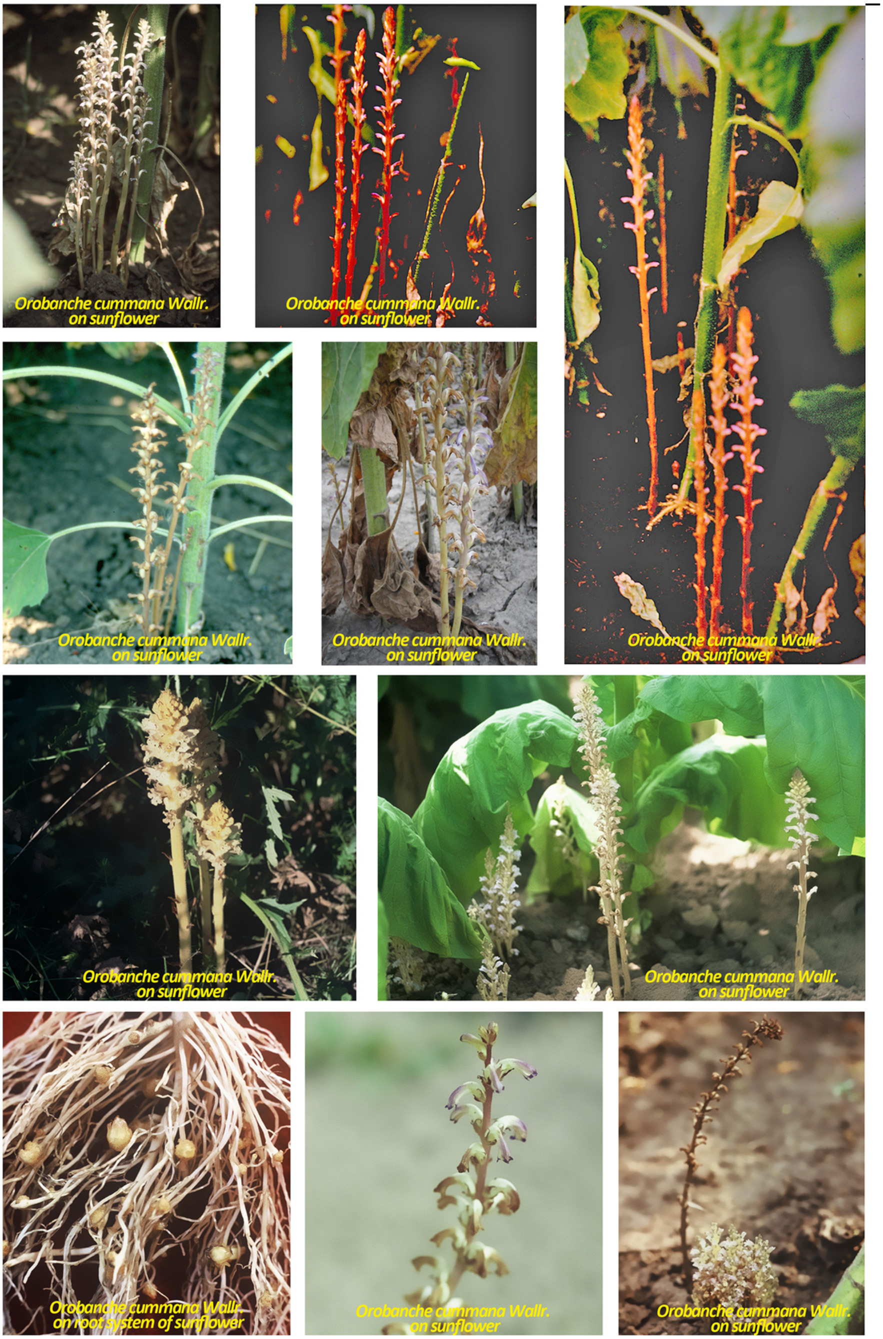

Figure 2: The morphological variability of Broomrape populations, which can be found in natural, field conditions on cultivated sunflower plants. (The authors thank Dr. Horvath Zoltan for permission to use Orobanche's photographs).

In an assessment of the broomrape emergence issue at the global level, Kaya (2014) stated the following.

In addition to the development of broomrape resistance genes, research should also be performed on altering the plant anatomy of plant organs, biochemical parameters (mechanical barriers, induced germination, hormones, etc.) aspects of the parasite side such as breeding system and genetics of virulence, to understand the dynamics of broomrape populations and race evolution. On the other hand, the Clearfield system is also an alternative and efficient control method with the use of imidazolinone (IMI) herbicide plus resistant hybrids. Combining resistance to herbicides with genetic resistance could provide a more horizontal and durable resistance and successful improvement of broomrape control in the future.

Fernandez-Martinez et al. (2012) concluded the following: In recent years, several research groups have made relevant contributions on breeding for resistance and development of control strategies. Following the identification and characterization of new virulent races, new sources and mechanisms of resistance have been developed and characterized at the genetic and molecular level. There have also been important contributions to the knowledge of the biology and genetics of the parasite that will contribute to understanding the dynamics of broomrape populations and race evolution.

Sunflower breeders and geneticists have achieved significant results in the use of molecular markers for identifying new broomrape races (A–F). Marker-assisted selection should be used even more in the future search for *Orobanche* resistance.

The sources of resistance to broomrape which have been discovered so far mostly use the gene of resistance taken from the wild species of the genus *Helianthus*. According to the results obtained so far, there are over 20 wild species of the genus *Helianthus* which contain the gene of resistance to broomrape. Some of the breeders who used wild species of sunflower for finding the gene of resistance to broomrape are Zhdanov (in the 1930s) – quotation Morozov (1947), Galina Pustovoit (1975), Škorić (1988, 1989, 2005), Christov et al. (1992, 1998, 2009), Jan et al. (2000, 2002), Jan and Fernandez-Martinez (2002), Fernandez-Martinez et al. (2000, 2007), Ruso et al. (1996), Sukno et al. (1998), and others.

The paper aims to show the dynamics of change in broomrape populations in Romania and Russia, as countries which have experienced the most prominent changes, as well as to point out certain dilemmas over the nomenclature of the new populations (races).

Material and method

Sunflower geneticists and breeders have been using different methods of testing resistance to broomrape (in open-field or greenhouse experiments, or through marker-assisted selection). Application of different methods often fails to provide confident results and adequate comparison of the obtained results.

The open-field method was used in the research, while trials were set up at the plots where a high emergence of broomrape plants had previously been observed.

Trials were set up in Romania and Russia, as countries which experienced frequent emergence of new broomrape populations (races).

Trials were set up in four Romanian locations (Cuza Voda, Crucea-Stupina, Braila-Valea Canepii and Tulcea-Agighiol) in 2014. The research included 390 sunflower genotypes and four controls (Performer, LC-1093, PR64LE20 and LG-5661) with a different degree of resistance. The main plot consisted of 2–4 rows in two replications. The final evaluation of resistance was performed during the stage of physiological maturity, while the number of broomrape-infected plants was determined at the main plot.

Ten hybrids with a different degree of broomrape resistance and five controls were studied in 2015. Unfortunately, hybrid LG-5631 was observed at only one locality. Trials were set up at five different localities in Romania. The main plot consisted of 2–4 rows. The final evaluation was carried out at physiological maturity by recording the number of broomrape plants at the main plot.

The study used the results obtained from only five hybrids (among the 10 tested hybrids) with a different degree of broomrape resistance.

The seed of the F_2 generation was produced in 2016, using only hybrid Hy-7 which exhibited complete resistance at all five localities.

The trial was set up at the experimental field of VNIIMK – Don trial station in Rostov on Don (Russia) during 2017. Besides hybrid Hy-7 (F_1) and the F_2 generation, the trial included four control hybrids (Donskoy-22, Bella, LG-5580, and PR66LE25). The trial was carried out in two replications, and the main plot consisted of two rows. The mixture of broomrape seed collected from Russia, Ukraine, Romania, Bulgaria, Turkey and Spain was incorporated into the plot used in the previous trials. Broomrape resistance was evaluated at physiological maturity, while the percentage of broomrape infested sunflower plants and the degree of infestation were calculated.

Results and discussion

The results obtained in four-year trials in different localities of Romania and VNIIMK – Trial station for breeding near Rostov on Don (Russia) revealed new variability in broomrape populations.

Trials carried out in 2014 at four localities in Romania (Cuza Voda, Crucea-Stupina, Braila-Valea Canepii, and Tulcea-Agighiol) revealed greater variability in

Table 1: The degree of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at various Romanian localities in 2014 (number of broomrape plants/main plot).

No.	Genotype	Locality			
		Cuza Voda	Crucea-Stupina	Braila-Valea Canepii	Tulcea-Agighiol
1	LC-1093 (rase F)	542	154	1.136	267
2	Performer	1.532	437	3.397	432
3	LG-5661	48	28	40	10
4	PR64LE20	69	58	129	43
5	No. 97	0	46	70	18
6	No. 144	0	72	4	9
7	No. 185	0	3	1	4
8	No. 194	0	32	130	25
9	No. 242	0	64	17	11
10	No. 341	0	30	123	35
11	No. 378	0	2	1	0
12	No. 388	0	110	63	10
13	No. 367	38	0	1	2
14	No. 143	–	38	0	14
15	No. 9	60	100	246	0
16	No. 188	20	8	1	0
17	No. 190	2	36	1	0
18	No. 191	3	33	5	0
19	No. 219	150	53	280	0
20	No. 274	40	40	28	0
21	No. 302	50	7	45	0
22	No. 348	1	9	3	0
23	No. 365	3	18	15	0
24	No. 381	8	23	4	0
25	No. 383	1	18	1	0
26	No. 384	2	7	2	0
27	No. 386	0	12	15	0

broomrape populations compared to race F at all localities, since control line LC-1093 exhibited susceptibility. Line LC-1093 showed the highest susceptibility at the locality Braila-Valea Canepii (Table 1), followed by the localities Cuza Voda, Tulcea-Agighiol, and Crucea-Stupina. Even the susceptible hybrid Performer exhibited a high degree of infection. Also, control hybrids LG-5661 and PR64LE20 resistant to race G showed a certain degree of susceptibility at all four localities. Different degrees of susceptibility or resistance was observed at the same localities in all 390 tested genotypes, while only the genotypes which showed resistance in at least one locality were taken for analysis. There were 10 genotypes at the locality

Cuza Voda which had complete resistance to broomrape, whereas in Crucea-Stupina, Braila-Valea Canepii and Tulcea-Agighiol, genotypes showed different degrees of susceptibility. The only exception was observed in genotype No. 378, which was resistant at two localities – Cuza Voda and Tulcea-Agighiol. Among all the tested genotypes, only the genotypes No. 367 and No. 143 showed complete resistance at localities Crucea-Stupina and Braila-Valea Canepii, respectively.

Complete resistance was exhibited by 15 genotypes at the locality Tulcea-Agighiol, while different degrees of broomrape infestation was observed in other localities. The exception was observed in No. 378 and No. 386, which were completely resistant at localities Tulcea-Agighiol and Cuza Voda.

Among the tested genotypes, the following ones arouse breeders' interest: No. 185, No. 378, No. 367, No. 365, No. 348, No. 383, and No. 384 (Table 1).

Ten new hybrids and four controls were tested at five Romanian localities in 2015, as well as one line (LC-1093 gene for race F) (Table 2).

Based on the obtained results, races (populations) which cannot be controlled by gene Or_6 have been confirmed. To examine 10 new hybrids, only five hybrids with a different degree of resistance to broomrape were chosen for analysis.

Hybrid Performer susceptible to broomrape was most attacked at the locality Ciresu-Braila. Hybrid LG-5542, resistant to race H, was under broomrape attack at two localities: Stupina-Constanta and Topolog-Tulcea, thus indicating the occurrence of new genetic variability in broomrape populations, which cannot be controlled by genes for race H.

Hy-7 was the only hybrid of all the tested ones which had complete resistance at all five localities, thus indicating that genes in hybrid Hy-7 can control new genetic variability of broomrape populations found in Stupina-Constanta and Topolog-Tulcea (Table 2).

The seed of the F_2 generation was produced in 2016, to further explore broomrape resistance genetics in the hybrid Hy-7.

Seeds of hybrid Hy-7 (F_1) and its F_2 generation were tested in 2017, at plots infested with broomrape from Russia, Ukraine, Romania, Bulgaria, Turkey, and Spain. The trial was carried out at experimental fields of VNIIMK – Don trial station in Rostov on Don (Russia). Control hybrids were included in this trial: Donskoy-22 (susceptible control), Bella, LG-5580, and PR66LE25.

The obtained results showed the following degrees of broomrape infestation in susceptible hybrids: Donskoy-22 100%, Bella 30%, LG-5580 23%, PR66LE25 19.4%, Hy-7 17.1%, and its F_2 generation 35.1% (Table 3).

The results revealed that hybrid Hy-7, which contained resistance genes above race H and exhibited complete resistance in 2015 at five Romanian localities, was infested with broomrape in 17.1% sunflower plants under the given

Table 2: The degree of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at various Romanian localities in 2015 (number of broomrape plants/plot).

No.	Genotype	Localities									
		Ciresu-Braila		Iazu-Ialomita		Stupina-Constanta		Topolog-Tulcea		Viziru-Braila	
		Sunflower plants/plot	Broomrape plants/plot	Sunflower plants/plot	Broomrape plants/plot	Sunflower plants/plot	Broomrape plants/plot	Sunflower plants/plot	Broomrape plants/plot	Sunflower plants/plot	Broomrape plants/plot
1	Performer	89	679	48	97	39	102	46	169	45	204
2	LC-1093	47	41	43	8	32	41	43	51	46	19
3	LG-5542	45	0	–	–	38	5	46	6	46	0
4	LG-5631	46	12	–	–	–	–	–	–	–	–
5	PR64LE20	48	16	44	0	41	18	44	26	44	0
6	Hy-1	128	887	95	136	47	68	95	113	105	38
7	Hy-2	120	798	105	87	55	95	95	233	94	98
8	Hy-7	126	0	95	0	44	0	96	0	141	0
9	Hy-9	124	0	106	1	55	11	82	18	100	8
10	Hy-10	132	2	94	0	46	16	93	59	95	30

Table 3: Evaluation of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at the infested plot: VNIIMK – Don trial station, Rostov on Don in 2017.

No.	Genotype	Percentage of infested sunflower plants/main plot	Degree of infestation
1	Donskoy-22	100	∞
2	Bella	30	3,0
3	LG-5580	23	1.0–1.2
4	PR66LE25	19.4	2.6
5	Hy-7 (F_1)	17.1	1.1
6	F_2 (Hy-7)	35.1	2.0

conditions. The results indicate new variability of broomrape populations at that locality.

The results of Gorbachenko et al. (2018) suggest the possibility of developing new cultivars of sunflower resistant to the new variability of broomrape populations.

This method of testing broomrape resistance has its drawbacks due to the differences in broomrape distribution at the testing plots. Also, broomrape expression depends on several factors, such as soil type, distribution and amount of rainfall during the vegetation period, temperature, pH value, and so forth.

The obtained results are not precise; they are rather general results which provide a perspective on the varying degrees of resistance.

Based on exhaustive research, we can accept with high certainty the resistance genetics for races A, B, C, D, E and F with secure differential lines. However, speaking of the new races G and H, we might not be completely sure. The question is whether the same mutation can occur in broomrape populations, in various countries during the same year? Certain differences which have not yet been studied in detail surely exist. The rapid spread of the new races is also notable. In response to this question, Kaya (2014) concluded that the new races increase each year by 50% more than in the previous year. In the period from 1995 to 2002, the infested areas increased from 2 to 35%, leading to the conclusion that new *Orobanche* races occupied more than 60–70% of the sunflower production area in the Trakya Region in 2002.

The question regarding the degree of virulence in the new broomrape populations remains unanswered. Significant differences have best been illustrated by Pacureanu-Joita et al. (2012), stating that the most aggressive populations were found in Moldova, Romania, Russia, and Turkey.

When it comes to testing the variability of broomrape populations at the molecular level, we can conclude that a great number of authors have dealt with this problem. The results of Perez-Vich et al. (2004) who analyzed the resistance of the line P-96 to races E and F at the molecular level. Based on a linked map comprising 103 marker loci distributed on 17 linkage groups, it was determined that only five QTLS ($Or_{1.1}$, $Or_{3.1.}$, $Or_{7.1}$, and $Or_{13.2}$) were responsible for resistance to race E, while only six QTLS ($Or_{1.1}$, $Or_{4.1}$, $Or_{13.1}$, $Or_{13.2}$, and $Or_{16.1}$) controlled resistance to race F and they were found on seven of the 17 linkage groups. The results suggest that sunflower resistance to broomrape is controlled by a combination of qualitative, race-specific resistance affecting the presence or absence of broomrape and quantitative, non-race-specific resistance affecting the number of broomrape stalks per plant.

New studies worth mentioning are the ones carried out by Duca et al. (2017), who performed molecular characterization of 39 broomrape populations from Moldova using SSR markers.

Based on the obtained results, the authors concluded that some populations from the Southern region (especially Carabetovca, Alexanderfeld, Stefan-Voda, and Slobozia Mare) have shown major differences in the obtained profiles and presented a high degree of genetic variability. The study revealed the genetic diversity of *O. cumana* populations and contributed to research by providing useful information on this economically important pathogen.

Given the different degrees of virulence observed at trial localities, the results confirm the necessity for a detailed molecular analysis of different broomrape populations found in many Romanian localities.

The results of Gorbachenko et al. (2018) offer the possibility of developing sunflower hybrids resistant to the new variability of broomrape populations which cannot be controlled by genes for race H.

In conclusion, the results obtained in this research partly differ from the results obtained by Rişnoveanu et al. (2016), which was to be expected since the trials were carried out at various localities (plots). However, the results concur with authors' conclusion, confirming the identification of new races of *Orobanche* previously undetected at the trial localities.

Broomrape control through development of hybrids resistant to herbicides

For over 15 years, all large breeding companies and public institutions have been breeding sunflower hybrids for resistance to imidazolinone herbicides, thus developing mass production of dominant hybrids resistant to herbicides in countries with frequent broomrape occurrence. Many companies combine genes of resistance to broomrape (Or_5 and Or_6) with genes of resistance to imidazolinone herbicides, thereby providing secure, long-term hybrid resistance.

At the beginning of the twenty-first century, public resistance sources were found in the wild sunflower *Helianthus annuus* L. discovered in Kansas (USA). Resistance source named CHLA-PLUS, obtained by Sala (2008) through induced mutations, has lately been used by the company BASF.

It has been shown experimentally that the gene CHLA-PLUS a higher degree of IMI-resistance than the gene Imr_1, Imr_2. Breeding centres wishing to use the CHLA-PLUS gene for breeding purposes have to sign a contract on its use with the company BASF. At the same time, BASF provides a protocol for screening for resistance at the molecular level (CLEARFIELD® Protocol SF30).

Broomrape can also be managed by the development of IMI-resistant hybrids of by using biological control measures. In parallel with the search for broomrape resistance genes, efforts should be made to after the anatomy of plant organs as well as biochemical parameters (mechanical barriers, germination inhibitors, phytoalexins, etc.).

Conclusion

Based on the studies in sunflower genetics and breeding for broomrape resistance in the Black Sea area, especially in Romania and Russia, the following conclusions can be made:

There is permanent combat between breeders and geneticists on one side, and *Orobanche* on the other, and the WINNER is often found at different sides;

Methods have been developed for the evaluation of resistance to *Orobanche*;

Conventional methods and MAS (Marker Assisted Selection) have been developed in breeding for resistance to *Orobanche*;

In Romania, a new virulent population (races) of broomrape have emerged, especially in the regions of Braila, Tulcea, and Constanta. At certain localities, new variability of broomrape populations has been observed, which cannot be controlled by genes for race H;

Likewise, at the trial station of VNIIMK – Don Trial Station in Russia, a new virulent broomrape population emerged, which cannot be controlled by genes for race H. Luckily, sunflower breeders have developed new genotypes resistant to the new variability of broomrape populations;

There is a permanent change in populations of *O. cumana* Wallr., thus hindering the success of breeding for resistance;

Sources of broomrape resistance genes are found in certain wild species of *Helianthus*, which are transferred after detection to the elite lines of cultivated sunflower using interspecies hybridization;

Besides control through genes for broomrape resistance, *Orobanche* can successfully be controlled by the development of IMI-resistance hybrids.

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